Environmental Toxicology and Pharmacology 55 (2017) 165–174

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Research paper

Imidacloprid insecticide exposure induces stress and disrupts glucose homeostasis in male rats

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ARTICLE INFO

Keywords:
Forced swim test
Glucose transporter
Imidacloprid
Neonicotinoid
Open field test

ABSTRACT

In the present study, we evaluated the stress response in adult rats who were administered imidacloprid (IMI) orally in two doses (0.5 and 1.0 mg/kg bw for 60 days). It led to an alteration in the levels of cortisone and catecholamines and induced behavioral deficits, particularly in the animals exposed to the dose of 1.0 mg/kg. IMI was further analyzed for the effect on glucose homeostasis in developing and adult rats at a dose of 1.0 mg/kg bw where it elicited a hyperglycemic effect. Moreover, we observed an alteration in the mRNA levels of glucose transporters. Histopathological and immunohistochemical data displayed structural perturbations in pancreatic tissue with a decline in the expression of insulin and GLUT4, particularly in the developing rats. Collectively, IMI treatment resulted in stress represented by behavioral and biochemical changes, particularly at a dose of 1.0 mg/kg bw. Moreover, IMI perturbed the glucose regulation through hyperglycemic activity in both developing and adult rats, an observation clearly evident in the developing rats.

1. Introduction

In pets, the control of ectoparasites, such as ticks, lice, and flea, is indispensable for the prevention of various vector-borne diseases leading to high morbidity and in some cases, mortality. As a consequence, the control of infection by ectoparasites in dogs and cats has become a major concern (Chomel, 2011). Imidacloprid (IMI) is an ectoparasiticide member belonging to a novel family of compounds known as neonicotinoids that share structural similarities to nicotine. IMI has been reported to be employed as a veterinary medicinal product since 1991. In addition, it is extensively applied for the treatment of seeds in agriculture for the protection of crops against termites, piercing–sucking pests of tea, grains, vegetables, and cotton (Schenker et al., 2003).

IMI is a systemic neurotoxic insecticide characterized by extremely selective toxicity for insects over vertebrates. It interferes with the synaptic transmission of stimuli in the central nervous system (CNS) of the insects (Tomizawa and Casida, 2005). It performs this function by acting as an agonist at the postsynaptic nicotinic acetylcholine receptor (nAChR), especially the α4ß2 subtype, leading to a blockage in the nicotinergic neuronal pathway and accumulation of acetylcholine, consequently resulting in neurotoxicity in the insects. On the other hand, mammalian nAChRs exhibit a lower binding affinity for IMI than in insects. Therefore, the acute oral toxicity of IMI in mammals is considered moderate (Yamamoto et al., 1995). With respect to the above-mentioned information, neonicotinoids are the largest distributed and marketed insecticides in more than 120 countries (Tomizawa and Casida, 2003); the distribution represents approximately 17% of yearly overall sales of insecticide compounds in 2006 (Nauen et al., 2008). In 2010, the global annual production of IMI was reported to be 20,000 tons (Simon-Delso et al., 2015).

IMI has been detected in the global environment, where it is reported to accumulate in soils and sediments. It also exhibits a high runoff and releasing potential to surface and groundwater (Bonmatin et al., 2015) because of its high persistence in the water samples and its property to not get readily biodegradable in the aquatic environments (Tišler et al., 2009). According to the US Geological Survey (2003), its concentrations in the surface waters were recorded up to 14 µg/L. Furthermore, it tends to persist in crops, vegetables, and fruits, thereby extensively exposing non-target animals and humans to neonicotinoids including IMI.

Despite the lower toxicity of IMI to mammals than to invertebrates, many investigators have demonstrated it to be toxic to vertebrates, both aquatic and terrestrial (Mineau and Palmer, 2013). With respect to

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http://dx.doi.org/10.1016/j.etap.2017.08.017
Received 24 April 2017; Received in revised form 8 August 2017; Accepted 17 August 2017
Available online 24 August 2017

terrestrial organisms, the toxicological analyses on this pesticide on human peripheral blood lymphocytes, rat bone-marrow cells, and lymphocyte culture of rabbits have exhibited potential genotoxicity (Demsia et al., 2007; Stivaktakis et al., 2016). Also, IMI was reported to induce an oxidative stress in male mice (El-Gendy et al., 2010); it was found to be hepatotoxic and led to impairment in the male reproductive system of rats (Arafat et al., 2014; Lonare et al., 2014; Mohamed et al., 2017). In white Leghorn cockerels, it resulted in immunotoxic effects (Balani et al., 2008); endocrine disruption of the hypothalamic–pituitary–thyroid (HPT) axis in wildlife birds (Pandey and Mohanty, 2015); and neurobehavioral deficits in rat pups (Kara et al., 2015). IMI-treated seed ingestion produced lethal effects on adult partridges and led to a reduction of both breeding investment and offspring immunity (Lopez-Antia et al., 2015). In addition, a few cases of fatal intoxication in the humans following ingestion of 10% IMI insecticide formulation have been recorded (Shadnia and Moghaddam, 2008; Iyyadurai et al., 2010). Hence, based on the experimental studies, it has been listed as a Class II chemical (moderately hazardous) by the World Health Organization (World Health Organization, 2009).

Biomonitoring markers have witnessed an expanding interest with respect to their employment in providing measurements and estimating biological exposure to the toxins. To accomplish this objective, several end points are utilized as an indicator for the extent of pollutant exposure to stress. Stress is known to induce changes in various physiological conditions, with a potential to result in pathological states (Chrousos, 1997). The responses are thought to be a consequence of a complex interaction of stress and altered activity of several mechanisms, such as domains in central neurotransmitters, neurohormonal factors (Herman and Cullinan, 1997), and increment in free radical generation and oxidative damage in the CNS (Wang et al., 2007). Hence, the primary aim of the present study was to monitor the toxicological stress response of IMI at various doses by assessing the levels of cortisone and catecholamine (indicators of exposure to stressful conditions) in adult rats, with a focus on behavioral impairments.

Recently, the rate and prevalence of diabetes have risen steeply. It has been demonstrated that the effect of environmental pollutants, for example, food contaminants, plasticizers, pesticides, and organic metallic compounds may contribute to the development of conditions predisposing an individual to diabetes (Alonso-Magdalena et al., 2010). The change in enzyme activity in the metabolic pathways, such as glycolysis, glycogenesis, glycogenolysis, and gluconeogenesis; glands, such as adrenal gland; and oxidative stress induction in pancreas, muscle, and liver were considered responsible for the disturbing impact on glucose homeostasis (Rahimi and Abdollahi, 2007). Thus, the present study also proposed to evaluate the alterations in the glucose homeostasis induced as a consequence of IMI exposure to developing and aged rats.

2. Materials and methods

2.1. Tested chemicals

Analytical standard, purity grade (100%) imidacloprid ($C_9H_{10}ClN_5O_2$, CAS No. 138261-41-3) was purchased as Pestanal® (a registered trademark of Sigma-Aldrich Laborchemikalien GmbH; Germany). IMI was suspended in dimethyl sulfoxide (DMSO) and orally administered to rats throughout the experimental periods in the both experiments.

2.2. Animal grouping and treatment

Twelve developing and 30 adult male Sprague–Dawley rats (28 days and 3 months old, respectively) were utilized in the experiment. These were obtained from the Laboratory Animal farm, Faculty of Veterinary Medicine, Zagazig University and acclimated to the laboratory environment for 14 days where the animals were housed in stainless-steel cages, maintained in a controlled environment at a temperature range of 21–24 °C and humidity between 50 and 60% with a 12 h light/dark cycle. The rats were provided diet and water *ad libitum* throughout the study period. Individual body weights were determined weekly during dosing and were used for dose calculation. Animal care and experimental protocols were approved by Ethics of Animal Use in Research Committee (EAURC), at Zagazig University, Egypt.

After the exposure, animals fasted for 6 h and the blood was rapidly collected from median canthus (orbital vessels) of control and treated rats where the serum was separated by centrifugation at 3000 rpm for 10 min and stored at −20 °C for further biochemical analyses. All rats of each group were then weighed for calculation of gain in body weight and euthanized by cervical dislocation under sodium pentobarbital anesthesia (60 mg/kg). Tissue specimens were collected and fixed in neutral buffered formalin for histopathological investigation. In addition, the specimens were dissected and rinsed with sterile physiological saline (0.9%, NaCl). Subsequently, the samples were snap frozen by immersion in liquid nitrogen and preserved at −80 °C for further gene expression analysis.

2.3. Toxicological stress–response study in adult rats (dose–response)

For the stress–response experiment, three groups of adult male rats (n = 6) were administered orally by gavage with 0.5 and 1.0 mg/kg bw/day IMI, while the control group was treated with DMSO as a vehicle for a period of 60 days. The treatment doses were selected to be lower than the reported non-observable effect level, which is 5–10 mg/kg bw/day in rats (Kara et al., 2015).

2.3.1. Behavioral evaluations

In order to evaluate the behavioral alterations provoked by IMI intoxication, all behavioral tests were carried out in the same experimental testing room on the last day of the experimental period. Rats were transferred to the testing room in their home cages, and remained about 30 min to accommodate prior to testing. The experimenter was blind to the treatments of the animals. Behavioral analyses like open field test, forced swim and swimmer performance tests were performed in the different experimental groups as follows.

2.3.1.1. Open-field test. The open-field test was conducted in a square plastic box measuring 72 × 72 × 30 cm; the rats were placed individually at the center of the open field arena and behavioral parameters were monitored for 5 min, starting 2 min after placing the animal in the test cage. The open field apparatus was then thoroughly cleaned with 5% ethanol before placing the next rat to preclude the possible cueing effects of odors left by previous rats. The behavioral parameters observed were ambulation and locomotor activity (the number of floor sections entered by both feet), rearing frequency (the number of times the animal stood on its hind legs), grooming frequency (the number of grooming movements), and immobility (freezing) duration (total time in seconds without spontaneous movements). The open field was divided into two squares, and the number of entries and time spent in the central area were recorded. This provided a measure of anxiety-like behavior (Cont'o et al., 2005), as it was considered to be an unprotected area for rats. The control and the experimental rats were intermixed for the observations, which were recorded between 10:00 and 12:00 a.m.

2.3.1.2. Forced swim and swimmer performance tests. Each animal was forced to swim in a cylindrical glass tank (50 cm in height, 25 cm in diameter) loaded with 35 cm of 25 ± 1 °C water cylinder for a 3-min test. Here, the total immobility, struggling, and swimming times were recorded (Brotto et al., 2001). Swimming performance test was conducted by placing each animal at the center of the glass aquarium and was observed for 5–10 s. The swimming performance was rated according to the position of nose and head (angle) on the surface of the